

# Assessment of Various Biomasses in the Removal of Phenol from Aqueous Solutions

Senem Ustun Kurnaz<sup>1</sup>, Hanife Buyukgungor<sup>2\*</sup>

<sup>1</sup>General Directorate Of Turkish State Hydraulic Works, 7.Regional Directorate, Planning Department, 55209, Samsun, Turkey

<sup>2</sup>Environmental Engineering Department, Faculty of Engineering, Ondokuz Mayıs University, Samsun, Turkey

## Abstract

Recently, bioadsorbents have emerged as an eco-friendly effective and low cost material option. These bioadsorbents include some fungus, agricultural wastes, algae and bacteria.

Biosorption attracts attention at recent years as an alternative to conventional methods for phenolic compounds removal from water and wastewater. Fungal cell walls and their components have major role in the biosorption. Fungal biomass can also take up considerable quantities of pollutants from aqueous solutions by adsorption or a related process, even in the absence of physiological activity.

This study, investigated the use of non-viable pretreated cells of *Aspergillus niger*, *Rhizopus arrhizus* and activated sludge to remove phenol from aqueous solutions in batch reactors. Three types of died pretreated *Aspergillus niger*, *Rhizopus arrhizus* and Activated sludge biomasses powders were used as a biosorbent to remove phenol present in an aqueous solution at a concentration of 50 mg/l. It was observed that initial pH, initial biosorbent concentration and adsorption time affected adsorption rates.

It was observed that, sulfuric acid-pretreated died *Aspergillus niger* biomass powder was the most effective in three types of used microorganisms to remove phenol. The maximum removal of phenol was observed at in an initial pH of 5 for the sulfuric acid-pretreat biomass. Approximately, 85-90 % phenol was removed for the sulfuric acid pretreated *Aspergillus niger* biomass and an initial concentration of 50 mg/l of phenol within 50 minutes.

## Introduction

Phenols are commonly occurring industrial pollutants (Guerin, 1998). Phenolics are present in the wastewaters of industries such as cooking, synthetic rubber, pharmaceuticals, oil and gasoline, paper, textiles and wood etc. (Rao and Viraraghavan, 2002). Nearly all phenols are toxic and some are known to be human carcinogens, and if they enter food chain, important environmental problems can result. They are present in different concentrations in this wastewaters. Their concentrations typically range from 100 to 1000 mg/L (Molina et al., 2003). The United States Environmental Protection Agency (EPA) regulates lowering phenol content in the wastewater to less than 1mg l<sup>-1</sup> from the several thousand mg/L (Denizli et al., 2004).

However, the impurities, especially phenol, should be removed from the wastewater. Because the phenol would effect to the environment. Fish and aquatic life were also affected by phenol

at 5-10 mg/L concentration. The phenol could be formed carcinogen with chlorine in the environment (chloro-phenols compounds) at the chlorine concentration of 0,002 mg/L (Sirianuntapiboon et al., 1999).

Methods for the removal of these compounds use activated carbon adsorption, solvent extraction and chemical oxidation (Denizli et al., 2005; Syamsiah and Hadi, 2004). Traditionally, activated carbon adsorption is the most widely used technique for the removal of phenols and their derivatives. The high cost of activated carbon has stimulated interest to use cheaper raw materials. Polymer based adsorbents are widely employed for the removal of phenols, but the high cost of polymers has also stimulated interest in examining the feasibility of using cheaper adsorbents. Recently, microorganisms have been considered as one of the most promising adsorbents (Denizli et al., 2004).

Biosorption process offers the advantages of low operating costs, regeneration of biosorbent, minimization on the volume of chemical and/or biological sludge to be disposed of and high efficiency in detoxifying very dilute effluents (Bahadir et al., 2007; Qodah, 2006).

Biosorption is a property of certain types of inactive, dead, microbial biomass to bind and concentrate contamination from even very dilute aqueous solutions (Büyükgüngör et al., 1996). Biomass exhibits this property, acting just as a chemical substance, as an ion exchanger of biological origin. It is particularly the cell wall structure of certain algae, fungi and bacteria which was found responsible for this phenomenon.

In the concept of biosorption, several chemical processes may be involved, such as adsorption, ion exchange, and covalent bonding. The biosorptive sites on the microorganisms are carboxyl, hydroxyl, sulphhydryl, amino and phosphate groups (Denizli et al., 2004). Fungal cell walls and their components have a major role in biosorption. Fungal biomass can also take up considerable quantities of organic pollutants from aqueous solution by adsorption or a related process, even in the absence of physiological activity (Denizli et al., 2004; Gadd and White, 1989).

**\*Corresponding author:** Hanife Buyukgungor, Environmental Engineering Department, Faculty of Engineering, Ondokuz Mayıs University, Samsun, Turkey, Tel: (362) 312 19 19/1220-1221.; E-mail: [hbuyuk@omu.edu.tr](mailto:hbuyuk@omu.edu.tr)

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The main aim of this research was to determine the effects of different parameters (types of microorganisms, pretreatment of biomass, pH, biomass concentration) which were effective at the removal of phenol from aqueous solutions using non-living biomass of *Aspergillus niger*, *Rhizopus arrhizus* and activated sludge by biosorption.

## Materials and Methods

### Microorganisms and growth conditions

A fungal strain of *A. niger* (RSHMB-04017) was inoculated into a liquid media comprised of dextrose (20 g/L); peptone (10 g/L); and yeast extract (3 g/L).

*R. arrhizus*, a filamentous fungus, was obtained from the USA Department of Agriculture Collection (NRRL 2286). *R. Arrhizus* was grown at room temperature in liquid media containing malt extract (17.0 g/L) and soybean peptone (5, 4 g/L).

The activated sludge used in this came from a local sewage treatment plant (Samsun, Turkey). The activated sludge was inoculated into a growth medium comprised of glucose (10 g/L), (NH<sub>2</sub>)<sub>2</sub>CO (2,62 g/L), DAP ((NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>) (0,85 g/L) and MgSO<sub>4</sub>·7H<sub>2</sub>O (0,05 g/L) in distilled deionized water. The flasks were placed on a rotary shaker operating at 125 rpm. The activated sludge thus cultured aerobically.

### Preparation of the microorganism for biosorption

All culture work was conducted under aseptic conditions at room temperature. After the growth period, *Aspergillus niger*, *Rhizopus arrhizus* and Activated sludge biomasses was washed twice with deionized water to remove the growth medium sticking on to its surface. *Aspergillus niger*, *Rhizopus arrhizus* and Activated sludge live pellicles were pretreated in three different ways:

- Autoclaved for 15 min at 121°C and 124 kPa.
- Immersed in a 0,1 M solution of sulfuric acid for 1 h; washed thoroughly with deionized water; and subsequently autoclaved for 15 min.
- Immersed in a 0,1 M solution of sulfuric acid for 1 h; washed thoroughly with deionized water; and subsequently autoclaved for 15 min.

The biomasses obtained after pretreatment was spread on glass petri dishes and dried at 70 °C in an oven for a period of 48 h. The dried biomass was powdered using a pestle and a mortar. Dried biomasses was homogenized in a mixer to destroy cell aggregates and sieved at Fristsch sieve. Different particle ranges of biomass, such as <45, 45–63, 90–125, 125–250 and 250–500 µm were used for this phenol biosorption study.

### Biosorption studies

The pretreated biomasses (50 mg and 45-63 µm) were added to erlenmeyer flasks containing 100 mL phenol solution (50 mg/L). Batch kinetic studies were conducted with pretreated biomasses at room temperature to determine the equilibrium time. The pH of the solution was 5. The flasks were covered with parafilm to prevent the loss of phenol by volatilization. The flasks were agitated at 125 rpm on a shaker (Nüve ST 402) for 180 min. Samples were with drawn at regular intervals of time up to

5-10 min. After he samples were filtered by Whatman (pore size 45 µm). The filtrate was analyzed by the chloroform extraction method for phenol as per the (APPHA, AWWA, WEF Standards methods, 1998) by using spectrophotometer.

Phenol uptake (q) was determined as follows:

$$q = \frac{V(C_0 - C_f)}{m} \quad (1)$$

Where q (phenol uptake, mg/g) is amount of phenol adsorbed on the biosorbent, V (mL) the volume phenol containing solution in contact with biosorbent, C<sub>0</sub> (mg/L) the initial concentration of phenol in the solution, C<sub>f</sub> (mg/L) the final concentration of phenol in the solution and m (g) is the dry weight of fungal biomass. These are schematically shown in Figure1.

The pretreated biomass providing the maximum removal of phenol at the unadjusted pH value (pH=5) was chosen for detailed pH studies. Initial pH values of 1, 2, 4, 5, 6, 8 and 10 were studied. The concentration of the biomass was 0, 5 g/L for the pH studies also. pH was adjusted to the acidic or the basic range using either 1 M H<sub>2</sub>SO<sub>4</sub> or 1 M NaOH.

In order to determine the effect of biomass concentration on phenol removal, Initial concentration of the biomass of 0.1, 0.2, 0.3, 0.4, 0.5 and 1 g/L were studied. Initial pH value was optimum pH for biomass studies also.

## Results and Discussion

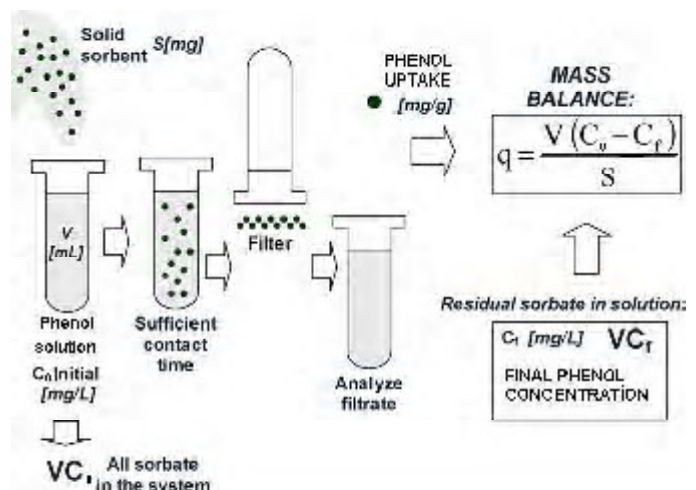
### Biosorption time for different pretreated biomasses

All the systems using different pretreated *A.niger*, *R. arrhizus* and activated sludge biomasses reached equilibrium in 50-70 min.

### Effect of types of microorganisms and pretreatment

The removal trend with the pretreated died *Aspergillus niger* biomasses was as follows: sulfuric acid pretreated *A. niger* (89, 2 %) > sodium hydroxide pretreated *A. niger* (69, 6 %) > autoclaved pretreated *A. niger* (53, 6 %).

The removal trend with the pretreated died, *Rhizopus arrhizus* biomasses was as follows: sulfuric acid pretreated *R. arrhizus* (51, 3 %) > sodium hydroxide pretreated *R. arrhizus* (43, 4 %) >



**Figure1:** Experimental set up for the removal of phenol from aqueous solutions (Vieira and Voleskym, 2000).

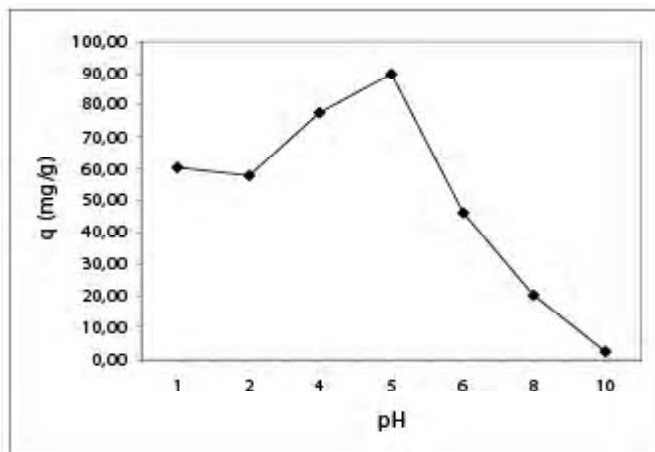
autoclaved pretreated *R. arrhizus* (42, 1 %).

The removal trend with the pretreated died, *activated sludge* biomasses was as follows: autoclaved pretreated activated sludge (33, 2 %) > sulfuric acid pretreated activated sludge (26, 2 %) > sodium hydroxide pretreated activated sludge (23, 0 %).

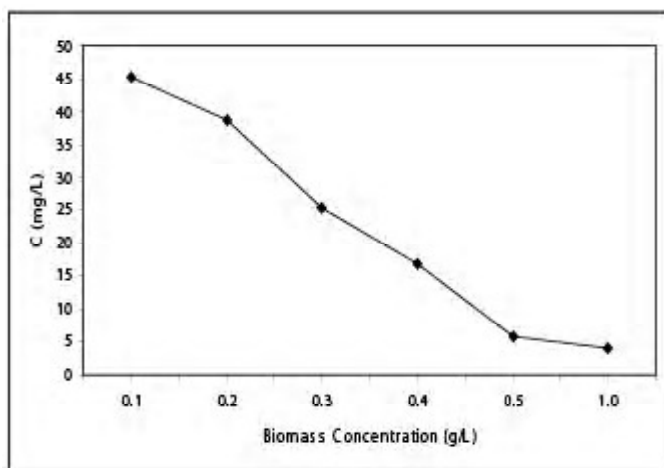
Sulfuric acid-pretreated died *Aspergillus niger* biomass powder was the most effective in three types of used microorganisms to remove phenol. The difference in the percentage phenol removal between  $H_2SO_4$ , NaOH and autoclaved pretreated *A. niger* biomass could be due to  $H^+$  ions contributed during  $H_2SO_4$  pretreatment.

Rao and Viraraghavan, (2002) have reasoned that Sulfuric acid-pretreated died *Aspergillus niger* was the most effective in five different pretreating ways (autoclaving,  $H_2SO_4$ , NaOH,  $HNO_3$ , laboratory detergent) remove phenol.

Pretreating *A. niger* with sulfuric acid could generate positively charged sites on its surface due to the sorption of an excess of  $H^+$  ions. Phenol being weakly acidic will be partially ionized in solution. These ions will be negatively charged and will be directly attracted due to electrostatic forces by positively charged fungal biomass surface. Unionized phenol molecules will also be attracted, possibly by physical forces Rao and Viraraghavan, (2002).



**Figure 2:** Effect of initial pH on phenol removal using sulfuric acid pretreated *Aspergillus niger* biomass from aqueous solution (phenol concentration, 50 mg/L; biomass concentration, 0, 5 g/L; contact time, 50 min).



**Figure 3:** Effect of biomass concentration on phenol removal using sulfuric acid pretreated *Aspergillus niger* biomass from aqueous solution (initial phenol concentration, 50 mg/L; pH, 5; contact time, 50 min).

### Effect of initial pH on phenol removal

The most important single parameter influencing the sorption capacity is the pH of adsorption medium. The initial pH of adsorption medium is related to the adsorption mechanisms onto the adsorbent surface from water and reflects the nature of the physicochemical interaction of the species in solution and the adsorptive sites of adsorbents (Bahadir et al., 2007; Büyükgüngör, 2000). Removal of phenol from aqueous solution was very sensitive changes in biosorption medium pH. To determine the effect of pH of the biosorption medium on the initial rates of phenol, the pH was varied between 1.0 and 10.0. Figure 2 shows the effect of initial pH on phenol removal using sulfuric acid pretreated *Aspergillus niger* biomass. The optimum initial pH of the biosorption medium was found to be 5 (Figure 2). The equilibrium phenol uptake ( $q$ ) was 89, 90 mg/g at this optimum pH value. An increase or decrease in the pH from this optimum pH resulted in a reduction in the biosorption of phenol.

These results were very similar to those obtained by (Fu and Viraraghavan, 1999) in their studies on the biosorption acid-blue-29 by sulfuric acid pretreated biomass and (Rao and Viraraghavan, 2002) in their on the biosorption phenol by sulfuric acid pretreated biomass (Ustün and Büyükgüngör, 2007).

### Effect of biomass concentration on phenol removal

In order to determine the effect of biomass concentration on phenol removal, Initial concentration of the biomass of 0.1, 0.2, 0.3, 0.4, 0.5 and 1 g/L were studied. For phenol adsorption using sulfuric acid pretreated *Aspergillus niger* biomass, as the concentration of microorganism was increasing at the medium, the phenol concentration was decreasing (Figure 3). At equilibrium conditions, biosorption amount of phenol was increasing parallel to biomass concentration. When the phenol remained on the solution concentration was investigated at each particle size, the optimum biomass concentration was obtained at 0, 5 g/L dose.

Actually, when initial concentration of the biomass of 1 g/L was studied, maximum phenol removal was obtained. But, it was determined that  $q$  (phenol uptake, mg/g) at 1 g/L biomass dose was lower than  $q$  (phenol uptake, mg/g) at 0.5 g/L biomass dose. The maximum  $q$  (mg/g) was obtained at 0.5 g/L biomass dose (Table 1). Thus, it can be stated as that the optimum biomass concentration was obtained at 0.5 g/L dose.

### Conclusions

It was observed that, under batch conditions, sulfuric acid-pretreated died *Aspergillus niger* biomass powder was the most

Biomass concentration (g/L)	Phenol concentration (mg/L)	$q$ (mg/g)
0,1	45,30	47,00
0,2	38,69	56,55
0,3	25,42	81,93
0,4	16,80	83,00
0,5	5,68	88,64
1	4,10	45,90

**Table 1:** Effect of biomass concentration on phenol removal using sulfuric acid pretreated *A. niger* (initial phenol concentration, 50 mg/L; pH, 5; contact time, 50 min).



effective in three types (*A.niger*, *R. arrhizus*, activated sludge) of used microorganisms to remove phenol. All the systems using different pretreated *A.niger*, *R. arrhizus* and activated sludge biomasses reached equilibrium in 50-70 min. The optimum initial pH of the biosorption medium was found to be 5. The equilibrium phenol uptake (q) was 89.90 mg/g at this optimum pH value. When the phenol remained on the solution concentration was investigated at each particle size, the optimum biomass concentration was obtained at 0.5 g/L dose.

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